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Original Research Article

Prediction of fatty acid and mineral composition of lentils using near infrared spectroscopy

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ABSTRACT

Lentils are an important source of both macro- and micronutrients. Their fat content is relatively low and is composed of mainly polyunsaturated fatty acids. The minerals found in lentils are mainly magnesium, potassium and iron. This study investigates the use of near infrared reflectance spectroscopy (NIRS) to predict the mineral content and fatty acid profile of lentil seeds (*Lens culinaris Medicus*). Samples (57) of brown, green, black and red lentils were analysed, and their mineral (calcium, iron and magnesium) and fatty acid contents were determined. NIR spectra for whole intact samples and after the samples were ground into powder were obtained, and the two recording methods were compared. The different compounds were predicted using the modified partial least squares regression method. The predictive models developed show excellent coefficients of determination (RSQ > 0.9) for the C 16:0, C 18:2, C 20:5n-3, C 21:0, omega 6 and calcium parameters. The results obtained reveal that it is possible to predict the fatty acid and mineral composition of lentils using near infrared spectroscopy. Furthermore, the results obtained show that the equations obtained can be applied to unknown lentil samples.

1. Introduction

Lentils (*Lens culinaris*) are of high nutritional value (Păucean et al., 2018); their macro- and micronutrient contents are very high, and they contain factors that encourage the bioavailability of minerals (Thavarajah et al., 2011b). Furthermore, they are suitable for celiac sufferers, are rich in fibre content, and contain compounds with antioxidant properties (Itagi and Singh, 2012). Their fat content is low, and their polyunsaturated fatty acids (PUFAs) are the most abundant group of fatty acids, followed by monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs) (Ryan et al., 2007). The concentrations of unsaturated and polyunsaturated fatty acids differ according to the lentil variety studied, although the major fatty acids never appear to vary (Zia-Ul-Haq et al., 2011; Gharibzahedi et al., 2012). Linoleic acid followed by oleic, palmitic and linolenic acids are the major fatty acids found in lentil oil (Gharibzahedi et al., 2012). Owing to their high unsaturated fatty acid content, lentils are considered to be a healthy food, and their consumption is associated with beneficial effects for diabetes, cardiovascular diseases, and some types of cancer (Faris et al., 2013).

As far as their mineral content is concerned, lentils contain minerals that are an essential part of our diet, with potassium, phosphorus, calcium, sodium, and magnesium being those found in the highest quantities. In terms of micronutrients, lentils also contain iron, zinc, copper and manganese (Gharibzahedi et al., 2012; Păucean et al., 2018). The great bioavailability of Fe in lentils means that they are considered to be an important food source for this mineral (Ariza-Nieto et al., 2007; Thavarajah et al., 2011b). However, the micronutrient content of lentils appears to vary greatly depending on their areas of cultivation. Thavarajah et al. (2011a) thus suggested that lentils cultivated in Australia, Nepal, and Canada are better sources of Se than those grown in Syria, Morocco, northwestern USA, and Turkey, with the prevailing temperature regime being the factor with the greatest influence on these variations (Thavarajah et al., 2011b).

The nutritional composition of lentils, together with the fact that they have interesting functional properties, has led the food industry to take an interest in this pulse; lentil flours and various texturized products have now reached the market. It would be very interesting for the food industry to rely on a tool that would allow the rapid estimation of the composition of the various compounds present in lentils. Near infrared spectroscopy (NIRS) technology has already been shown to be

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Table 1
Reference chemical data of the fatty acids (g 100 g^{-1} of total fatty acids) and mineral (mg 100 g^{-1} of seed), analyzed in the lentil samples for calibration and validation sets.

	Calibration set (N = 47)				External validation set (N = 20)			
Fatty acid*	Minimum	Maximum	Mean**	SD	Minimum	Maximum	Mean**	SD
C14:0	0.71	5.32	1.72	0.84	0.00	3.65	1.64	0.67
C15:0	0.00	2.30	0.27	0.35	0.00	0.52	0.20	0.11
C16:0	8.02	47.84	12.35	6.09	2.53	20.48	11.51	2.99
C16:1	0.00	1.93	0.39	0.38	0.00	1.00	0.32	0.23
C18:0	0.90	11.47	2.35	1.72	0.00	4.03	1.93	0.70
C18:1	12.69	38.10	19.85	4.84	7.96	30.67	19.32	3.79
C18:2n-6	6.58	48.13	39.49	7.74	17.24	62.66	39.95	7.57
C20:0	0.00	2.99	0.74	0.44	0.40	1.34	0.68	0.26
C20:1	0.00	11.62	1.11	1.99	0.34	1.24	0.57	0.32
C18:3n-3	0.99	27.84	18.71	5.37	2.87	34.68	18.77	5.30
C21:0	0.00	0.66	0.17	0.16	0.00	0.52	0.15	0.12
C22:0	0.00	2.94	0.79	0.55	0.00	2.15	0.74	0.47
C20:3n-6	0.00	1.36	0.20	0.23	0.00	0.52	0.15	0.12
C23:0	0.00	1.76	0.33	0.36	0.00	0.72	0.24	0.16
C20:5n-3	0.00	1.72	0.61	0.37	0.00	1.43	0.56	0.29
Σω6	7.17	48.90	39.93	7.76	17.59	63.18	40.39	7.60
Σω3	1.82	28.34	19.32	5.24	4.05	34.51	19.08	5.08
Mineral	**Minimum**	**Maximum**	**Mean**	**SD**	**Minimum**	**Maximum**	**Mean**	**SD**
Calcium	25.50	105.60	68.89	21.31	23.87	123.74	73.80	16.64
Iron	8.66	38.07	13.74	4.44	6.98	18.44	12.71	19.10
Magnesium	65.77	113.65	87.08	10.69	69.88	164.74	113.45	23.01

* C14:0 Myristic acid; C15:0 Pentadecanoic acid; C16:0 Palmitic acid; C16:1 Palmitoleic acid; C18:0 Stearic acid; C18:1 Oleic acid; C18:2n-6 Linoleic acid; C20:0 Arachidic acid; C20:1 cis 11 Eicosenoic acid; C18:3n-3 α linolenic acid; C21:0 Heneicosanoic acid; C22:0 Erucic acid; C20:3n-6 cis 8,11,14 eicosatrienoic acid; C23:0 Tricosanoic acid; C20:5n-3 Eicosapentaenoic acid.

** Average values obtained from the analysis of 57 samples of lentils, each sample was analysed in triplicate.

suitable for this purpose in other grain products, such as wheat grain, triticale, rye, barley, oats and sorghum (Salgó and Gerlgely, 2012; Caporaso et al., 2018), and is now being used by the food industry in online applications. In the case of lentils, NIRS has been shown to be suitable for assessing proximate composition (Moldovan et al., 2015; Revilla et al., 2019). However, to the best of our knowledge, the possibility of analysing minority compounds such as fatty acids or minerals has not yet been assessed. The objective of this study is to assess the viability of the use of NIRS to predict both the individual fatty acid composition and that of Σω-6 and Σω-3, together with the mineral, iron, calcium, and magnesium contents of lentils. To carry out this study, two recording methods were tested. First, the spectrum of whole lentils was recorded; subsequently, the lentils were ground into powder, and the spectrum was recorded again.

2. Materials and methods

2.1. Samples

A total of 57 samples of dried lentil (*Lens culinaris*) belonging to 8 different varieties were analysed. The group consisted of 47 samples of brown lentils, i.e., Armuña (35 from three different cultivars), Castellana (7) and Pardina (5), 4 samples of green lentil, i.e., Stones (2) and Du Puy (2), 3 samples of a black lentil, i.e., Caviar, and 3 samples of red lentils, i.e., Crimson (2) and Masoor Dal (1). The identity of the lentil varieties used in this project has been authenticated by the Legume Centre of Salamanca (Spain).

For each of the 57 samples analysed, 500 g of lentils was purchased in bulk from stores specializing in the sale of pulses. The samples were ground without removing their skin in a Foss Knifetec™ 1095 mill with the temperature controlled at 15 °C; they were passed through a 60 mesh sieve to obtain a uniform particle size.

2.2. Fatty acids

The lipids were extracted by means of the Soxhlet method using 10 g of ground lentils. The fatty acids were analysed by means of gas chromatography (GC) with an FID detector according to the method described by Lurueña-Martínez et al. (2010) after methylation of the sample with methanolic KOH. One microlitre of the sample was injected into the gas chromatography equipment (GC 6890 N, Agilent Technologies, USA) using a 100 m ×0.25 mm ×0.20 μm capillary column (SP-2560, Supelco, Inc., Bellefonte, PA, USA) equipped with a split/splitless injector and an FID detector. The injector and detector temperatures were 250 °C. The oven temperature program was initiated at 150 °C; the temperature was gradually increased by 1 °C/min to 165 °C, then by 0.20 °C/min to 167 °C, and finally by 1.50 °C/min to 225 °C. This final temperature was maintained for 15 min. The fatty acids were identified according to their retention times by comparison with commercial standards (Larodan Fine Chemicals AB, Malmo, Sweden). The quantification was expressed as g per 100 g of total fatty acid methyl esters.

2.3. Mineral composition

The calcium, iron and magnesium contents were determined by using an Ultima 2 ICP-OES (inductively coupled plasma optical emission spectrometry) equipment (Jobin Yvon, Paris, NJ, USA) according to the method described by González-Martín et al. (2015). Prior to the analysis of the mineral elements, the samples (0.2 g of ground lentils) were mineralized in a microwave system (Ethos Sel, Ontario, ON, Canada). HNO_3 (5 mL) was added, and a power of 1000 W was applied for 5 min. Then, an additional 5 mL of HNO_3 and 1 mL of H_2O_2 (30 %) were added, applying a power of 1000 W for 10 min. The sample was cooled to room temperature and prepared to 100 mL with distilled water. The quantification was carried out by using certified standard solutions (Scharlab, Spain); the results were expressed in mg kg^{-1} of lentils.

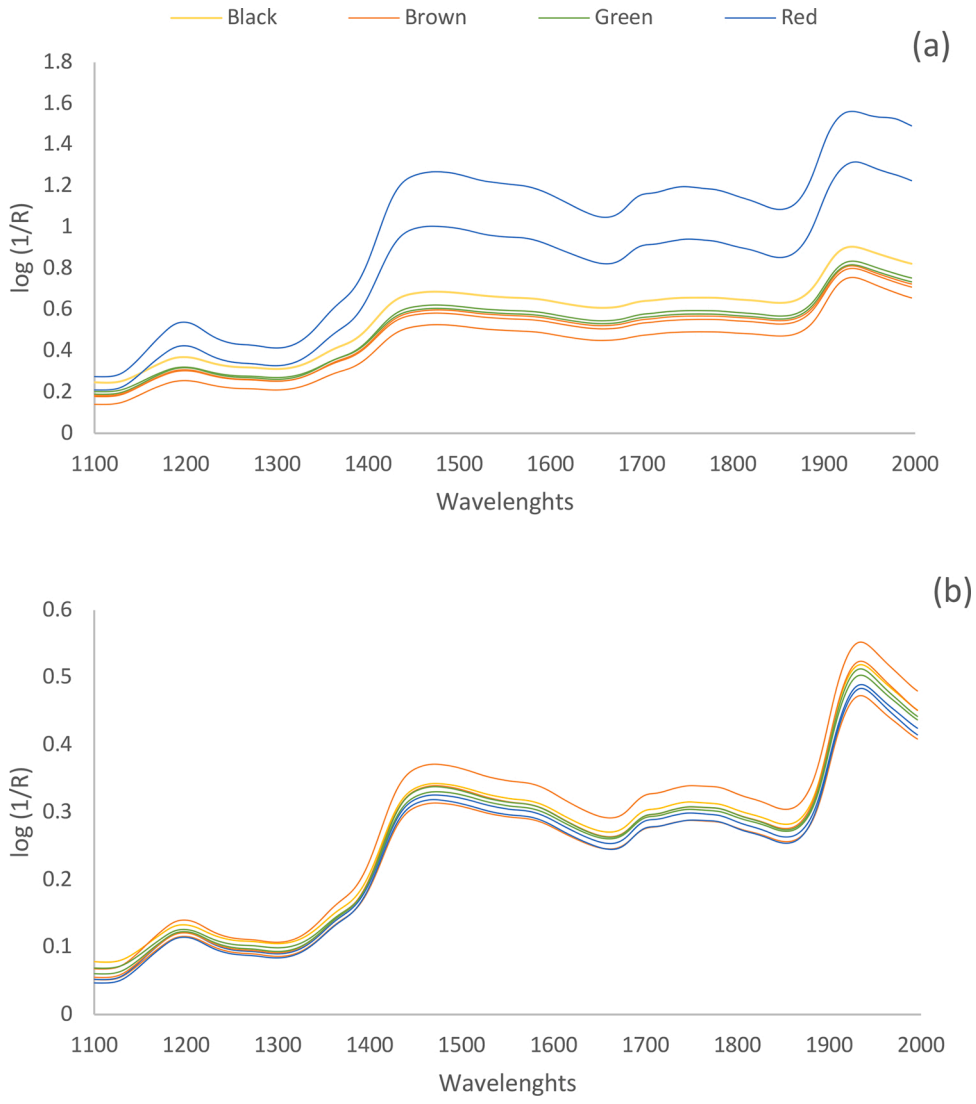

Fig. 1. Near Infrared average spectra, without pretreatment, of each lentil variety: (a) whole beans and (b) ground beans.

2.4. NIR spectroscopy

The NIR measurement of the lentil samples was obtained by using a Foss NIR System equipment with a spectral range of 1100−2000 nm, a remote reflectance probe, and a 5 cm × 5 cm window surface area. Two methods were compared to obtain the NIR spectrum of the lentils: on the one hand, the whole lentil sample was measured, and on the other hand, the lentils were ground in a Foss Knifetec™ 1095 mill with the temperature controlled at 15 °C. In both cases, the measurement was taken by the direct application of remote reflectance optic fibre.

2.5. NIR-chemometric methods

The 57 lentil samples analysed were divided into two groups at random; 47 samples were used for the development of the calibration (calibration set), and 10 samples were used for the external validation (validation set). The samples for each of these groups were chosen at random. Initially, a principal component analysis (PCA) was used. The criterion used to remove samples was the Mahalanobis distance (limit value H = 3). Different treatments, such as multiplicative dispersion correction (MSC), standard normal variant (SNV), DeTrend (DT) or SNV-DT, were tested separately to eliminate dispersion effects. In addition, different mathematical treatments were used to develop NIRS calibrations using a 2,4,4,1 type code in which the first digit was the number of the derivative, the second was the interval over which the derivative was calculated, the third was the number of data points in an average or smoothing, and the fourth was the second smoothing. Of all the treatments tested, the best one was chosen to calibrate each parameter independently. Using the $T \geq 2.5$ criterion, samples presenting high residual values when they were predicted were eliminated from the set. The statistical parameters of the calibration were obtained for each of the components after removing the samples for spectral (*H* criterion) or chemical (*T* criterion) reasons.

The mineral composition data (analysed by ICP) and the fatty acid composition data (analysed by GC) together with the spectral data obtained from the NIR recording of the samples were used for the development of calibration models using the modified partial least squares method (MPLS).

The models developed were assessed by analysing the ratio performance deviation (RPD), which can be defined as the relationship between the standard deviation of the chemical method (SD ref) and that of the root mean square error of prediction (SEP) in the NIR model and the mean square error of cross-validation (SECV), which is the combination of the validation errors generated among the various groups used in the cross-validation. The calibration equation was chosen based on the RSQ determination coefficient and the root mean square error of calibration (RMSEC).

Table 2
Statistical descriptors of calibration by NIR of the compounds studied according to the recording mode of lentil samples.

Component	Recording mode	Math treatment*	N	Mean	SD	Est. Min	Est. Max	SEC	SECV	RSQ	RPD
C14:0	Whole lentil	Detrend only 1,4,4,1	40	1.50	0.40	0.30	2.70	0.23	0.30	0.66	3.8
	Ground lentil	SNV only 2,8,6,1	44	1.64	0.67	0.00	3.65	0.28	0.53	0.83	3.4
C15:0	Whole lentil	None 1,4,4,1	41	0.20	0.12	0.00	0.55	0.07	0.12	0.65	5.9
	Ground lentil	SNV only 2,4,4,1	42	0.20	0.11	0.00	0.52	0.05	0.08	0.78	8.8
C16:0	Whole lentil	None, .10,10,1	37	11.06	1.60	6.27	15.86	0.43	1.31	0.93	15.6
	Ground lentil	Standard MSC 2,4,4,1	44	11.51	2.99	2.53	20.48	1.17	2.20	0.85	4.2
C16:1	Whole lentil	None 0,0,1,1	36	0.27	0.16	0.00	0.74	0.07	0.16	0.82	3.5
	Ground lentil	SNV only 1,4,4,1	42	0.32	0.23	0.00	1.00	0.13	0.20	0.67	2.7
C18:0	Whole lentil	Detrend only 2,10,10,1	37	1.90	0.65	0.00	3.86	0.35	0.58	0.72	4.1
	Ground lentil	Standard MSC 2,4,4,1	41	1.93	0.70	0.00	4.03	0.40	0.59	0.67	3.4
C18:2n-6	Whole lentil	SNV only, .4,4,1	36	41.29	4.13	28.88	53.69	2.19	3.55	0.72	3.2
	Ground lentil	Standard MSC 2,10,10,1	35	39.95	7.57	17.24	62.66	2.15	2.83	0.92	3.9
C20:0	Whole lentil	None 2,10,10,1	30	0.67	0.26	0.00	1.46	0.10	0.22	0.85	4.9
	Ground lentil	Standard MSC 2,4,4,1	42	0.68	0.24	0.00	1.41	0.09	0.19	0.85	4.9
C20:1	Whole lentil	None 2,10,10,1	38	0.56	0.32	0.00	1.51	0.17	0.31	0.71	12.4
	Ground lentil	Standard MSC 2,8,6,1	40	0.57	0.31	0.00	1.50	0.17	0.30	0.70	13.2
C22:0	Whole lentil	Detrend only 2,8,6,1	40	0.69	0.42	0.00	1.93	0.26	0.37	0.61	2.3
	Ground lentil	SNV only 1,4,4,1	43	0.74	0.47	0.00	2.15	0.30	0.45	0.59	2.0
C20:3n6	Whole lentil	Standard MSC 0,0,1,1	39	0.14	0.09	0.00	0.42	0.06	0.08	0.62	4.6
	Ground lentil	Standard MSC 2,4,4,1	43	0.15	0.10	0.00	0.45	0.04	0.08	0.87	7.6
C20:5n-3	Whole lentil	SNV only2,4,4,1	39	0.58	0.33	0.00	1.58	0.17	0.24	0.73	2.2
	Ground lentil	Standard MSC 2,4,4,1	43	0.56	0.29	0.00	1.43	0.09	0.24	0.90	2.7
Σn-6	Whole lentil	Standard MSC 1.4.4.1	36	41.83	4.00	2.98	53.83	2.20	3.54	0.70	2.6
	Ground lentil	Standard MSC 2,10,10,1	45	40.39	7.60	17.59	63.18	2.21	2.96	0.92	3.8
Ca	Whole lentil	None 2,4,4,1	42	73.80	16.64	23.87	123.74	54.84	145.09	0.98	4.3
	Ground lentil	None 2,10,10,1	46	68.56	21.42	4.283	132.85	110.78	128.40	0.73	1.9
Fe	Whole lentil	Standard MSC 1,4,4,1	37	12.72	1.91	6.98	18.44	10.91	15.48	0.67	4.3
	Ground lentil	Detrend only 2,10,10,1	42	12.72	1.79	7.32	18.11	10.47	14.68	0.66	4.6
Compounds that it was possible to calibrate only in ground lentils											
C18:1	Ground lentil	None 2,4,4,1	41	19.32	3.79	7.96	30.67	2.16	3.04	0.68	2.0
C18:3n-3	Ground lentil	Detrend only 2,4,4,1	44	18.77	5.30	2.87	34.68	2.55	3.94	0.77	2.2
C23:0	Ground lentil	Standard MSC 2,4,4,1	43	0.24	0.16	0.00	0.72	0.06	0.16	0.87	7.2
C21:0	Ground lentil	Detrend only 2,4,4,1	44	0.15	0.12	0.00	0.52	0.03	0.11	0.93	5.2
Σn-3	Ground lentil	None 2,4,4,1	44	19.28	5.08	4.05	34.51	2.76	3.77	0.70	2.2
Mg	Ground lentil	Detrend only 2,10,10,1	42	87.34	9.50	58.83	115.84	40.82	63.29	0.82	2.8

N: number of samples after removing the outliers; MSC: Multiplicative Scatter Correction. SNV; standard normal variate; SD: standard deviation; RSQ: multiple correlation coefficients; SEC: standard error of calibration; SECV: standard error of cross-validation; RPD: ratio performance deviation.

* The first digit is the number of the derivative, the second is the gap over which the derivative is calculated, the third is the number of data points in a running average or smoothing, and the fourth is the second smoothing.

3. Results and discussion

3.1. The fatty acid and mineral composition of lentils

The fatty acid and mineral composition of the 57 lentil samples (divided into calibration and external validation sets) is shown in Table 1. A total of 15 fatty acids were identified in the samples analysed by GC. The main fatty acids found in lentil samples were linoleic, palmitic, oleic and linolenic acids. These results agree with those obtained by other authors from different varieties of lentils (Ryan et al., 2007; Zia-Ul-Haq et al., 2011; Gharibzahedi et al., 2012; Zhang et al., 2014; Paucean et al., 2018). Other fatty acids detected in the samples were stearic, cis 11 eicosenoic and miristic acids. Owing to the high concentration of linoleic acid, PUFAs were the major group of fat compounds in the lentils tested, and the SFA contents were the minor group. The ω-6:ω-3 ratio, between 1.67 and 6.65, is in accordance with the average values described by Paucean et al. (2018) for red and green lentils. Regarding the mineral content, calcium, phosphorus and magnesium have been described as the minerals present in the largest proportion in lentils (Padovani et al., 2007). The samples analysed in this study (Table 1) showed a high variability in the concentration of the minerals found: calcium (25.0–105.60 mg 100 g^{-1}), magnesium (65.77–113.65 mg 100 g^{-1}) and iron (8.66–38.07 mg 100 g^{-1}). These results are in variance with those found in the literature for different lentil genotypes. The calcium content is lower than that described for cultivars grown in Pakistan (116−121 mg 100 g^{-1}) and Iran (168−170 mg 100 g^{-1}) (Zia-Ul-Haq, et al., 2011, Gharibzahedi et al., 2012; Ganesan and Xu, 2017). The iron and magnesium contents found in this study are higher than those described for lentils grown in Canada (Thavarajah et al., 2009, 2011b), Pakistan (Zia-Ul-Haq, et al., 2011) and Iran (Gharibzahedi et al., 2012).

The variability of the results obtained for the different parameters makes them suitable for use as reference values for the development of an NIR method for calibration purposes.

3.2. Spectral information and NIR calibration equation

Fig. 1 presents the average spectra of each of the 8 varieties of lentils (whole and ground) analysed in this study. The spectra of whole lentils (Fig. 1a) showed significant differences between the various varieties, with red and black lentils having higher absolute values. Spectral information from ground samples showed great homogeneity in both the pattern and in the absolute values of absorbance (Fig. 1b). In any case, the absorbance values of ground samples were lower than those found for whole lentils. The main absorption bands for both whole and ground lentils were located at 1476 nm, 1732 nm and 1924/1944 nm.

Calibrations were performed on both whole and ground lentil samples using MPLS. For this purpose, we used the chemical data obtained in the laboratory, and the absorbance of the samples from 1100 to 2000 nm was recorded every 2 nm. The absorbance was calculated as log 1/R (in which R is the value of the spectral reflectance obtained from the recording of the samples). To develop the calibration model, the outliers were eliminated using the Mahalanobis criterion and the T criterion, which resulted in a number of samples (N) for each parameter, as shown

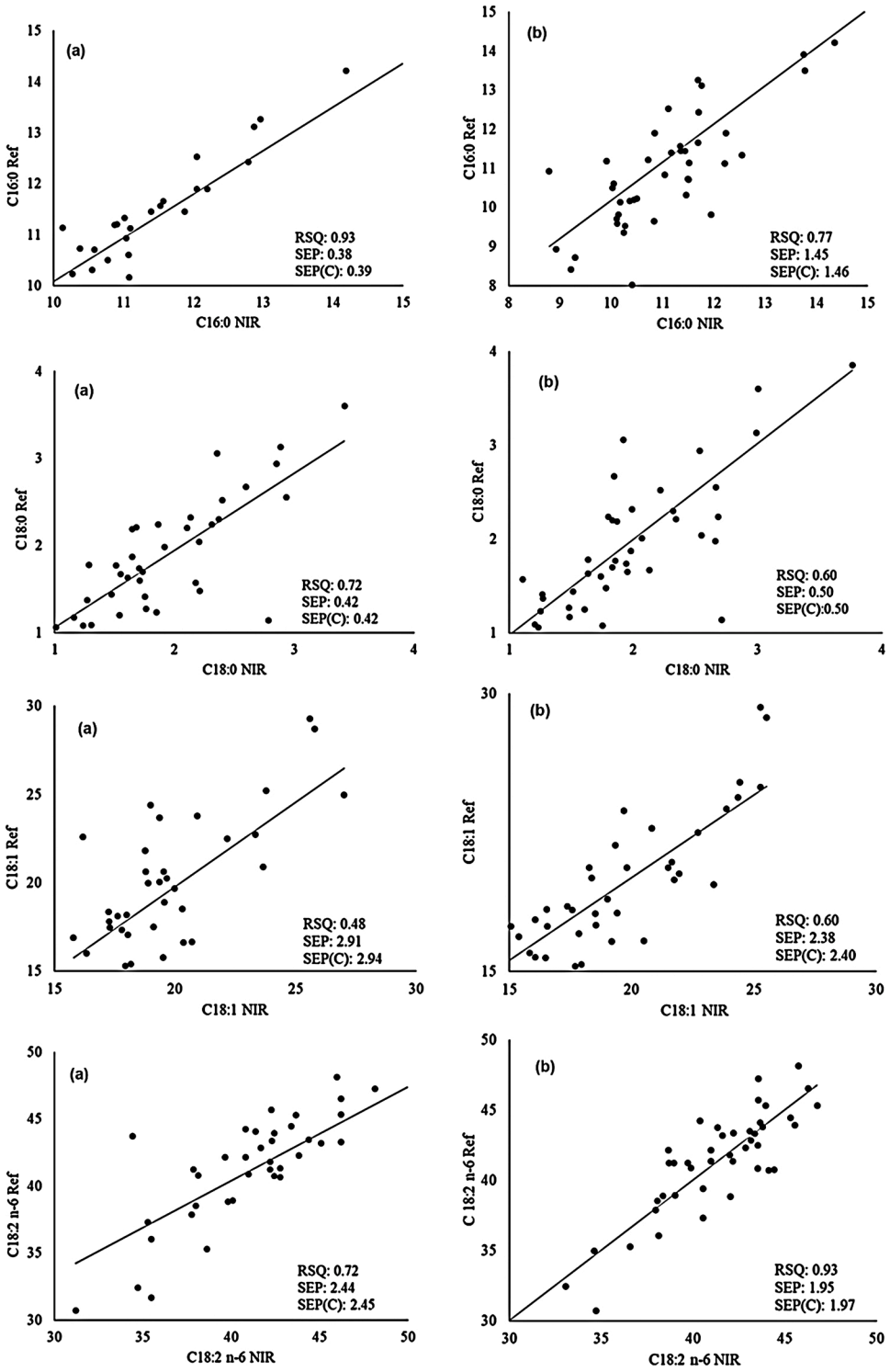

Fig. 2. A comparison between the reference values (Ref.) and the values predicted by the NIRS model for the main fatty acids found in lentils. The results correspond to the cross-validation, for both types of recording mode on the samples: whole lentils (a) and ground lentils (b).

in Table 2. This table also reflects the mathematical treatments applied and the statistical descriptors obtained in the calibration. Although all the parameters analysed in this study are minority compounds, with concentrations below the recommendations of NIRS of 100 mg 100 g^{-1} (Tremblay et al., 2009), the results show that it is possible to determine the fatty acids (C14:0¸C15:0, C16:0, C16:1, C18:0, C18:2n-6, C20:0, C20:1, C22:0, C20:3n6, C20:5n-3, and Σω6) in both ground and whole lentils. The acids C18:1, C18:3n-3, C21:0, C23:0 and Σω3 could only be estimated from the records collected from the ground samples. Regarding the mineral content, we were able to determine calcium and iron in both whole and ground samples. Magnesium, on the other hand, could only be determined in ground samples. The components with the highest RSQ (RSQ $>$ 0.9) showed a higher variability in the lentil samples analysed (the highest standard deviation values in Table 1). These results agree with those of Goi et al. (2019), who pointed out that high ranges of variability give rise to better prediction models.

The most suitable spectral pretreatment for calibration varied depending on the parameter analysed. Variations in particle size affect

the calibration statistics (Lovett et al., 2005), and it is for this reason that the optimization of mathematical treatments to obtain the calibration equations is different in samples of ground and whole lentils. The scattering effects are reduced in ground samples (Barbin et al., 2018), which could explain why some parameters could only be estimated from the ground samples. From Table 2, it can be seen that the multiplicative scatter correction (MSC) and the standard normal variate (SNV) treatments are those providing the best calibrations. Both are treatments that aim to minimize the effects of light scattering, which are mainly associated with physical factors such as particle size (Fearn et al., 2009).

One of the factors influencing the accuracy of NIRS equations is the range within which each component is found and its average concentration (Nie et al., 2008). According to our findings, linoleic and palmitic acids and Σω6 present the best results with RSQ > 0.9 and are at the same time the compounds with the highest concentration. However, other components that are present in very low concentrations, such as eicosapentaenoic and heneicosanoic acids, show better results (RSQ > 0.9) than oleic (RSQ = 0.68), linolenic (RSQ = 0.77) and stearic (RSQ = 0.72) acids, which are present at higher concentrations. Our results show similar calibration statistics to those found for fatty acids in previous studies. In *Brassica Juncea,* Kaur et al. (2016) found better calibration results for oleic acid (RSQ = 0.83) but worse results for linoleic (RSQ = 0.88) and linolenic (RSQ = 0.25) acids. Similar results (RSQ > 0.9) were obtained for linoleic acid in olive oil (Mailer, 2004), almond flour (Fernández-Cuesta et al., 2007) and rapeseed (Kim et al., 2007). However, worse results have been described by Fernández--Cuesta et al. (2007) for palmitic (RSQ = 0.62) and stearic acids (RSQ = 0.52). The SEVC errors are within the range found in previous studies for fatty acid calibration (Kaur et al., 2016).

The NIR regression models are made suitable by using the correlation between the concentration of each quantified parameter and the measurement at different wavelengths. The spectral information identifies a number of characteristic absorption bands. The C—H bond, which is an essential constituent of fatty acid molecules, absorbs strongly at wavelengths close to 1200, 1400, 1750, 2310 and 2340 nm. Absorption between 1786−2000 nm is related to the C—H first overtone of fatty acids (CH3- and −CH−CH-) (Shenk et al., 2007). Absorption between 1923 and 1961 nm is related to the O—H first overtone (Bazoni et al., 2017); at 1725 nm, the band absorption has been associated with oleic acid (Hourant et al., 2000), while the spectral region of 1450−1470 nm has been associated with H-bonds from OH groups (Rambo et al., 2020). Cruz-Tirado et al. (2020) associated the band absorption at 1715 nm with fat and fatty acids and the spectral region of 1900–1950 nm with moisture content.

For the mineral composition, Ca showed higher RSQ values (0.93) and Mg showed an intermediate value (0.82), while Fe showed a low RSQ value (0.67). These results match those obtained by Cozzolino and Morón (2004) in studies carried out on legumes in which they found low RSQ values for Fe (<0.7) and intermediate values for Mn (<0.8). The prediction results of Fe, Ca and Mg in the present study were mainly explained by their concentrations. The concentrations and range of values of Mg and Ca were relatively high compared with those of Fe (Table 1), which could explain why worse results were obtained in the prediction of Fe. Although Ca and Mg present similar values, the prediction of Ca is better (RSQ = 0.98 for whole lentils). Previous studies have shown that minerals may exist in different forms, some of which are easier to detect by NIRS (Tremblay et al., 2009).

The ability of NIR to determine the mineral composition is based on the fact that it is possible to establish a strong relationship between trace minerals and other constituents, mainly with O—H (water) bonds and with C—H combination bonds (functional organic groups) (Garnsworthy et al., 2000). Molecules that are active by infrared (IR) radiation are those that undergo modification at the dipolar moment during the transition. This means that the bonds that are commonly found in biological systems, such as C—H, O—H and N—H bonds, are active by IR. In our study, we found a strong correlation between Ca metal ions and wavelengths of 1500 and 1542 nm, between Fe with 1112 and 1228 nm and between Mg with 1530 and 1532 nm. In addition, all minerals analysed showed a correlation between their concentration and their absorbance in the 1510−1550 nm range.

Table 3
External validation (n = 20) with the chemical compounds properly calibrated by NIR.

Fatty acid	Recording mode	*p* (level of significance)	RMSE*
Ca	Whole lentil	0.311	15.33
	Ground lentil	0.203	14.97
Fe	Whole lentil	0.255	5.21
	Ground lentil	0.594	8.77
C14:0	Whole lentil	0.598	1.42
	Ground lentil	0.451	1.50
C15:0	Whole lentil	0.732	1.31
	Ground lentil	0.494	0.35
C16:0	Whole lentil	0.733	1.50
	Ground lentil	0.625	1.25
C16:1	Whole lentil	0.359	0.68
	Ground lentil	0.296	1.63
C18:0	Whole lentil	0.254	2.05
	Ground lentil	0.236	2.65
C18:2n-6	Whole lentil	0.451	3.26
	Ground lentil	0.203	0.91
C20:0	Whole lentil	0.530	1.23
	Ground lentil	0.320	1.12
C20:1	Whole lentil	0.083	2.01
	Ground lentil	0.068	0.56
C22:0	Whole lentil	0.965	1.54
	Ground lentil	0.735	1.23
C20:3n6	Whole lentil	0.471	0.98
	Ground lentil	0.428	1.55
C20:5n-3	Whole lentil	0.688	2.69
	Ground lentil	0.592	1.89
Σn-6	Whole lentil	0.215	3.65
	Ground lentil	0.174	3.21
Compounds that it was possible to calibrate only in ground lentils			
C18:1	Ground lentil	0.269	3.12
C18:3n-3	Ground lentil	0.530	1.99
C23:0	Ground lentil	0.411	1.01
C21:0	Ground lentil	0.145	0.85
Σn-3	Ground lentil	0.416	1.99
Mg	Ground lentil	0.125	9.35

* RMSE: root mean square error.

The preparation of samples prior to the recording of their NIR spectra is another factor considered in this study, since previous research has shown that it may affect the performance of the models developed by NIRS (Kaewcheenchai et al., 2010). This is because particle size may introduce variation into the absorbance spectra and make analyses less accurate (Phetkaeo et al., 2012). The results show that the RSQ values obtained were between 0.61−0.93 for whole lentils and between 0.59−0.93 for ground lentils, with higher RSQ values obtained for fatty acids and minerals in the ground samples. The heterogeneity of the sample appears to affect the predictive capacity of the NIRs to a different extent depending on the compounds for which the model is developed. Thus, Guy et al. (2011) found better predictive models in lamb samples when these were ground before recording. Similarly, in a corn study, ground samples were more suitable for detecting infected corn seeds (Phetkaeo et al. (2012)). In the case of soil samples, the literature on whether it is necessary to pre-grind the samples is contradictory. Reeves et al. (2002) and Russell (2003) reported more accurate calibration in soil samples if they were previously ground. However, other studies obtained optimal results from unground samples (Chang et al., 2001; Shepherd and Walsh, 2002). Previous research on lentil samples (Revilla et al., 2019) showed that sample preparation prior to NIR recording had a strong influence on the predictive ability of the developed models. When whole lentil samples were recorded, good calibrations were obtained for the parameters of weight, total fibre and ash. However, it was necessary to grind the lentils before recording to obtain good calibration

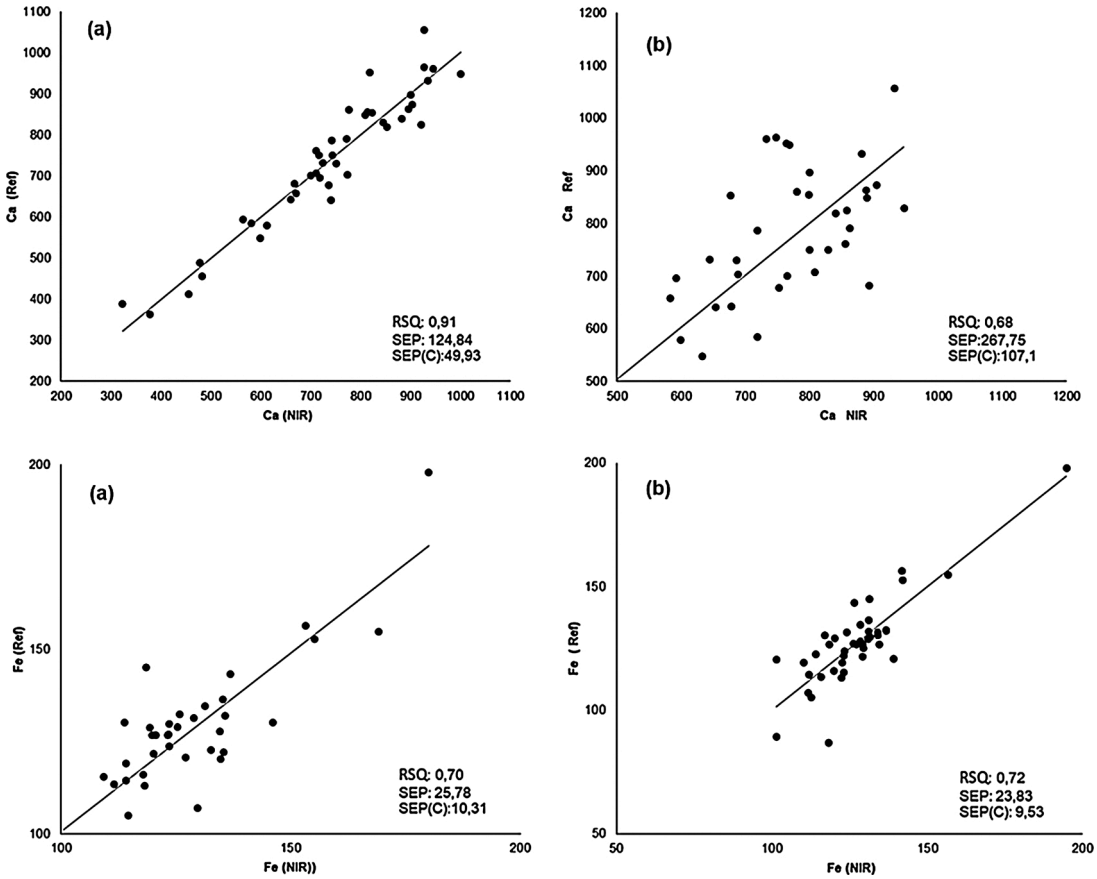

Fig. 3. A comparison between the reference values (Ref) and the values predicted by NIRS model for the minerals Ca, Fe and Mg, in lentils. The results correspond to the cross-validation, for both types of recording mode on the samples: whole lentils (a) and ground lentils (b).

models for the parameters of size, moisture and total fat.

3.3. Validation

Full cross-validation was used to validate the calibration models (internal validation), and the final model was used to predict the attributes in an independent set of samples (external validation). Six cross-validation groups were used in the internal validation, and the data predicted from the NIR models were compared with the reference data. The correlation between the values predicted by the NIRS model in the internal validation and the values obtained in the laboratory (Ref) for the major fatty acids and minerals can be seen in Figs. 1 and 2, respectively. The predictive ability of the models was assessed on the basis of residual predictive deviation (RPD), which is the ratio between the standard deviation of the measured reference values and the SEP; the higher the RPD value, the higher the accuracy of the calibration model. The RPD results suggest that the predictive ability is excellent for 16 of the 21 parameters studied: Fe, Ca, C 23:0, C 20:3n-6, C 20:1, C 20:0, C 18:2, C 18:0, C 16:1, C 16:0, C 15:0, C 14:0, saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids and omega 6. In the remaining 5 parameters (omega 3, C 22:0, C 20:5n-3, C 18:3n-3 and C 18:1), acceptable predictive ability was observed. In all cases, the RPD values were higher than 2, which means that the models are applicable to unknown samples (Monazen et al., 2005).

For external validation, 20 different samples that had not been included in the calibration set were used. The results obtained for the *p* (significance level) and root mean square error (RMSE) values are shown in Table 3. The *p*-values for fatty acids ranged from 0.068 for C 20:1 to 0.733 for C 16:0. This means that there were no significant differences between the results obtained in the determination of the individual fatty acids by gas chromatography and by the use of NIR technology. As the RMSE values are between 0.85 for C 21:0 and 3.65 for ω-6, the models developed are satisfactory for all the fatty acids analysed.

Regarding the mineral content, the *p*-values ranged from 0.121 for Mg to 0.594 for Fe. As mentioned above, this appears to indicate that there are no differences between the analysis of these minerals by ICP and NIR spectroscopy. However, the RMSE values are very high (>5.21) for the three minerals analysed, with Ca having the highest RMSE (15.33). The value of this statistical parameter should be as low as possible and must be compared with the error made in the traditional method of analysis to ascertain whether it is an acceptable error. The determination of minerals using NIR spectroscopy is carried out indirectly through their association with organic molecules, since they are theoretically incapable of absorbing energy in the NIR spectrum (Tremblay et al., 2009). Together with their ability to create complexes from a wide variety of molecules, this may be the reason for the high errors (RMSE) found. Previous studies have shown that the NIRS prediction of minerals entails many difficulties. In studies carried out on alfalfa, Halgerson et al. (2004) obtained high accuracy for Ca, P, and K, low accuracy for Mg and S, and were unable to predict Na. Studies of yerba mate (Rosa et al., 2015) showed potential prediction for P (RSQ = 0.62), Fe (RSQ = 0.72) and Cu (RSQ = 0.89) and considered NIRS to be an inefficient technique for the quantification of K, Ca, Na, Mn and Zn (Fig. 3)

4. Conclusions

NIR technology is proven to be an effective tool for determining the fat fraction and mineral content of lentils. The models developed using the modified partial least squares regression (MPLS) method have been

shown to be suitable for the determination of iron, calcium, C 23:0, C 22:0, C 20:5 n-3, C 20:3n-6, C 20:1, C 20:0, C 18:3 n-3, C 18:2, C 18:1, C 18:0, C 16:1, C 16:0, C 15:0, C 14:0, saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, omega 6 and omega 3 in both whole and ground lentil samples. Milling of samples has been found to be a highly suitable pretreatment for the registration of the samples by NIRS, as it allows the development of valid models for a larger number of analytes. In general, the range of applicability of the calibration equations obtained by NIR with a fibre-optic probe is similar to that achieved by reference techniques (GC and ICP-OES) and is suitable for the content of these analytes in lentils. The models established by NIR technology for the determination of fatty acids and minerals can be applied to unknown samples. The advantage of this method is that it constitutes a nondestructive, fast and low-cost analysis.

Authorship statements

Cristina Lastras: Formal análisis, **M. Inmaculada González Martín**: Methodology, Writing- Reviewing and Editing, **Isabel Revilla**: Writing- Original draft, Investigation, **Ana M. Vivar-Quintana**: Conceptualization, Writing- Reviewing and Editing.

Declaration of Competing Interest

The authors report no declarations of interest

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